Discovery of Template-Dependent DNA Polymerase (DNA Polymerase I)

Paper: *Enzymatic Synthesis of Deoxyribonucleic Acid*
Authors: I.R. Lehman, Maurice J. Bessman, Ernest S. Simms, and Arthur Kornberg (1957)

1. Background and Objective

- The study aimed to identify and characterize an enzyme responsible for **DNA synthesis** in *Escherichia coli*.
- Earlier studies indicated an enzyme system incorporating deoxyribonucleotides into DNA, but **the exact mechanism and enzyme properties remained unknown**.

2. Key Findings

2.1 Enzyme Purification and Characterization

- The team successfully isolated and partially purified **a novel enzyme**, later named **DNA Polymerase I**.
- The enzyme was found to catalyze **template-dependent** DNA synthesis.

2.2 Reaction Mechanism

- The enzyme catalyzed **polymerization of deoxyribonucleotides** by adding them to the **3'-hydroxyl (-OH) end** of a growing DNA strand.
- The polymerization followed a strict **5' → 3' direction**.
- The reaction required:
  - **All four deoxyribonucleoside triphosphates (dNTPs)**—dATP, dGTP, dCTP, and dTTP.
  - **A DNA template** for complementary base pairing.
  - **Mg^{2+} ions** as a cofactor.
  - **A primer** (revealed in later studies).
- The process released **inorganic pyrophosphate (PPi)** as a byproduct.

2.3 Substrate Specificity

- The enzyme incorporated only **deoxynucleoside triphosphates (dNTPs)**.
- **Deoxynucleoside diphosphates (dNDPs) and ribonucleotides were inactive**, confirming specificity for DNA synthesis.

3. Experimental Approach

3.1 Extraction and Purification of the Enzyme

The enzyme was extracted from *E. coli* and **purified through multiple biochemical steps**, including:

1. **Cell Lysis and Crude Extract Preparation**

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    - *E. coli* cells were **grown in nutrient-rich media**, harvested, and disrupted using a **sonicator** to release cellular contents.
    - A **centrifugation step** removed cell debris, and the supernatant (soluble protein fraction) was collected for further processing.
2. **Fractionation Steps to Purify DNA Polymerase I**
    - **Streptomycin sulfate precipitation** → Used to selectively remove contaminating nucleic acids.
    - **Ammonium sulfate precipitation** → Concentrated the enzyme by selective precipitation of proteins.
    - **Alumina and DEAE-cellulose chromatography** → Used to separate **DNA polymerase I from DNases and other proteins**.
    - **Final product:** A partially purified enzyme fraction, which still contained trace amounts of DNases.

3.2 DNA Synthesis Assay

To confirm DNA synthesis, the researchers designed a **radiolabeled nucleotide incorporation assay**:

1. **Reaction Setup**
    - The reaction mixture contained:
        - Partially purified **DNA polymerase I**
        - A **template DNA molecule** (to test template-dependency)
        - **dATP, dGTP, dCTP, and radiolabeled dTTP (P^{32}-dTTP)**
        - **Mg^{2+} ions** as a cofactor
2. **Measurement of DNA Synthesis**
    - The reaction was incubated at 37°C.
    - Newly synthesized DNA was **precipitated using perchloric acid**, separating it from unincorporated nucleotides.
    - The **radioactive DNA fraction was quantified** to measure nucleotide incorporation.
3. **Template Dependency Test**
    - When the **template DNA was omitted**, no DNA synthesis occurred.
    - This **confirmed that DNA polymerase I was template-dependent** and could not synthesize DNA de novo.

4. Significance of the Discovery

- **First demonstration of template-directed DNA synthesis**, which was crucial for understanding DNA replication.
- Led to the identification of **DNA Polymerase I's proofreading activity** and the discovery of multiple DNA polymerases.
- Enabled **modern molecular biology techniques**, including:
    - **Polymerase Chain Reaction (PCR)**
    - **DNA sequencing**
    - **Genetic engineering**
- Kornberg was awarded the **1959 Nobel Prize in Physiology or Medicine** for this discovery.

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